

was adjusted to pH 7.4 via dropwise addition of 1M NaOH. The solution was then filtered through a 0.22 μ m syringe filter aseptically.

Printing Conditions

[0226] Polymer bio-ink and collagen containing MMP-activator were loaded into the relevant cartridges, connected to the bioprinter. Both polymer bio-ink and activator were connected to a 0.007" nozzle, operating in between 25 and 30 kPa.

[0227] The 3D structure of the assay was designed using ILS custom-made software. The 3D rectangular prism structure was comprised of a bottom gel layer with a length and width of approximately 4.5 cm and 150 μ m in thickness.

Structure Validation

[0228] The resulting hydrogel structures were imaged using a USB digital microscope.

Example 19—Fibroblast Culture inside the Collagen-Containing PEG Hydrogel

[0229] The polymer bio-ink with collagen was prepared by mixing Type I bovine collagen with 10 wt % PEG-Mal in PBS at 1:1 v/v ratio. The pH of the solution was raised to 7.4 through the dropwise addition of 1M NaOH solution. PEG-bis-thiol activator containing equimolar thiol to maleimide concentration was prepared in PBS. Harvested human lung fibroblast (MRC-5) cells were pelleted and re-suspended in the collagen-containing polymer bio-ink. PEG hydrogel was prepared by firstly transferring the polymer bio-ink into a well plate and followed by transferring an equal volume of the cell-containing activator into the same well. 100 μ L of DMEM+10 v/v % FBS was then added into each well and the samples was incubated for 6 days, with a bright field images taken at day 0, 3 and 6 to monitor cell activities.

[0230] Biological compatibility of the gel for fibroblast culture was assessed by looking at the change in fibroblast morphology from rounded morphology into spindle like morphology.

[0231] Those skilled in the art will appreciate that the present disclosure is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

[0232] The present disclosure also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps, features, compositions and compounds.

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1. A 3D printed hydrogel formed from a maleimide containing polymer cross-linked using a bis-thiol containing cross-linking agent having at least two thiol functional groups.
2. The 3D printed hydrogel according to claim 1, wherein the maleimide containing polymer is selected from the group consisting of maleimide containing polysaccharides, including polymers containing fructose, sucrose or glucose monomers; synthetic polymers, including poly(ethylene glycol) (PEG) maleimide, poly(hydroxyethyl methacrylate) (PHEMA) maleimide, poly(ϵ -caprolactone) (PCL) maleimide, poly(vinyl alcohol) (PVA) maleimide, poly(vinylpyrrolidone) (PVP) maleimide, poly(N-isopropylacrylamide) (NIPAAm) maleimide, poly(propylene fumarate) (PPF) maleimide, poly(ethyleneimine) (PEI) maleimide, poly(3-methacrylamidopropyl) trimethylammonium (PMAETMA) maleimide, poly(-lysine) (PLL) maleimide, poly(acrylic acid) (PAA) maleimide, poly(styrene sulfonate) (PSS) maleimide, poly(acrylic acid-stat-dimethylaminoethyl methacrylamide) (P(AA-stat-DMAEMA)) maleimide, and poly(arginine methacrylate) maleimide, or derivatives thereof; maleimide containing biopolymers, including gelatin maleimide, cellulose maleimide, hyaluronic acid maleimide and alginate maleimide; maleimide containing nucleobase polymers including maleimide containing polymers of adenine, thymine, guanine and/or cytosine repeating units; and any combination thereof.
3. The 3D printed hydrogel according to claim 2, wherein the maleimide containing polymer comprises a PEG maleimide.
4. The 3D printed hydrogel according to claim 2, wherein the maleimide containing polymer comprises a gelatin maleimide.
5. The 3D printed hydrogel according to any one of claims 1 to 4, wherein the bis-thiol containing cross-linking agent is selected from the group consisting of synthetic polymers, biopolymers, small molecules, bioactive molecules, and any combination thereof.
6. The 3D printed hydrogel according to claim 5, wherein the synthetic polymers are selected from the group consist-